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UNITED STATES PATENT APPLICATION

FOR

READING DEVICE, METHOD, AND SYSTEM FOR CONDUCTING LATERAL FLOW ASSAYS

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Background of the Invention

Membrane-based test devices, particularly devices used in diagnostic medicine, employ a variety of internal and external calibrators to provide a qualitative or a quantitative result for an analyte of interest in a test solution. One type of membrane-based test device is a lateral flow assay.

In general, lateral flow assays are membrane-based test devices in which a sample that is suspected of containing the analyte of interest is placed at or near one end of a membrane strip. The sample is carried to the opposite end of the membrane strip by a liquid phase that traverses the membrane strip by capillary action. While traversing the membrane strip, the analyte in the test sample, if any, encounters one or more "capture" reagents with which it may react to produce a detectable signal.

Home use assay devices such as pregnancy tests and the like are now well established. Home use assays may be intended to detect physiological changes in the human body, with the objective of promoting the health and well being of an individual. Consumers are becoming increasingly health conscious, and it is a significant advantage if the consumer is capable of monitoring his or her own bodily functions, including levels of hormones and the like.

There are many different assays that are indicative of physiological changes in the human body. Furthermore, there are many different assay devices that operate by reading an assay strip or test sample. Some devices use fluorescence emission, and others use light reflectance.

United States Patent No. 6,235,241 B1 to Catt et al. ("the Catt patent") is directed to an assay result reader used in conjunction with an assay device. A commercially available device similar to that shown in the Catt patent is known as a UNIPATH CLEAR PLAN Easy® Fertility Monitor. This device is shown in Figure **1** herein, and comprises a fertility monitoring device **21** with a removable hand held cover **22**, which fits into a receiver **23** upon the housing **25**. Bodily fluids are applied to the test strip **24**, and the test strip **24** may be placed into the receiver **23**, where the test strip **24** receives light that shines through a window **26** upon the test strip **24**. Then, the level of reflected light is analyzed to give a result.

One of the problems with fertility monitoring devices as described is that they are not capable of providing a high degree of sensitivity, in many instances. That is, some analytes need to be monitored for medical purposes, but do not require a high degree of sensitivity or a sophisticated instrument to detect accurately and precisely the levels of analyte. Many currently available home use reading devices have a low signal to noise ratio, which may be caused in part by the undesirable

introduction of excess amounts of stray or ambient light into the viewing window. In conducting precise measurements using a reflectance-based regime, it is critical that the amount of stray ambient light be reduced or eliminated to achieve a high degree of sensitivity. It is therefore highly desirable to maximize the signal to noise ratio, and increase the sensitivity of such reading devices.

Another reading device for home use is known as an ACCUCHECK® Blood Glucose Meter manufactured and distributed by Boehringer Mannheim Diagnostics of Indianapolis, Indiana 46250. The ACCUCHECK® device is a reflectance-based instrument designed for home use in checking blood glucose levels. The instrument does not employ a lateral flow assay. Instead, a user is instructed to place a drop of blood upon a test pad. The reflectance sensor portion of the instrument contains a removable holder, with two rectangular windows.

What is needed in the industry is a sensitive reading device designed for lateral flow assay test strips. A reading device that provides an efficient and reliable means for quickly placing a test strip into position to receive a reading or result, while avoiding excess ambient and stray light would be desirable. A reading device providing high sensitivity for detecting hormones and the like would be desirable. A reading device having a window that achieves a high degree of efficiency in the transmission and reflectance of light would be useful.

Summary of the Invention

In the invention, a reading device for lateral flow assays, and a system for conducting assays, may be provided. The reading device is configured for detecting an assay result from a membrane strip, in which the result is revealed by the binding of a detectable analyte within a detection zone along the membrane strip. The assay reading device comprises a housing and a receiving port within the housing. The receiving port may include a light barrier structure, and admits a membrane strip directly from the outside of the housing. That is, a membrane strip is inserted into the receiving port. The receiving port may be configured for minimizing the introduction of stray or ambient light into the reading device.

A reading mechanism also may be provided which includes a source of electromagnetic radiation, and one or more sensors capable of detecting the intensity of reflected electromagnetic radiation. The source of radiation and the sensors may be positioned within the reading mechanism so that when the membrane strip is admitted into the receiving port, the radiation impacts the detection zone upon the membrane strip prior to impacting the sensor.

In another embodiment of the invention, a test kit, including a lateral flow assay reading device and a porous liquid permeable membrane strip may be provided.

In yet another embodiment of the invention, a system for conducting a lateral flow assays may be provided for detecting the quantity of analyte that resides in a test liquid. The system may include a probe configured for generating a detectable signal, and a membrane strip designed for mobilizing a test liquid. The membrane strip includes a detection zone. Furthermore, a reading device as previously described is employed, with a receiving port and light barrier structure configured for minimizing stray light into the reader. An assay result having increased sensitivity is achieved by way of the invention.

Brief Description of the Drawings

A full and enabling disclosure of this invention, including the best mode shown to one of ordinary skill in the art, is set forth in this specification. The following Figures illustrate the invention:

Figure **1** is a perspective view of the CLEAR PLAN EASY® Fertility Monitor previously discussed;

Figure **2** is a perspective view of one embodiment of the reading device of the invention, showing the light barrier structure and receiving port;

Figure **3** shows a perspective view of the reading device in which the receiving port **45** has been exploded upwards to reveal details;

Figure **3a** is a view of the underside of the top plate, showing interaction of the pressure plate with the top plate in the receiving port;

Figure **4** shows a cross sectional view of the receiving port in one embodiment of the invention, as taken along line **4-4** of Figure **2**;

Figure **5** shows an alternate embodiment of the reading device of the invention having a channel on the upper surface of the reading device configured to receive a membrane test strip;

Figure **5a** shows a cross sectional view of the membrane strip receiving portion of the reading device as taken along lines **5a-5a** in Figure **5**;

Figure **5b** shows a design layout for the electronics of the reading device, including a microcontroller, LCD display, and the like;

Figure **6** shows a closer view of the membrane strip receiving portion of the embodiment previously shown in Figure **5**, showing one particular application in which the membrane strip includes a nub that interlocks into one or more notches; and

Figure **7** shows a cross sectional view of the structure shown in Figure **6**, as taken along lines **7-7** in Figure **6**.

Detailed Description of the Invention

Reference now will be made to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not as a limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in this invention without departing from the scope or spirit of the invention. For instance,

features illustrated or described as part of one embodiment can be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention cover such modifications and variations as come within the scope of the appended claims and their equivalents.

In the invention, an optical reflectance meter or reading device is provided. The reading device may be used with lateral flow assays to provide quantitative results. The metering device may be designed to provide improved sensitivity and increased accuracy. The method and system of the invention may serve as a more accurate and sensitive alternative to direct visual examination of a membrane assay strip.

The reading device of the invention may include various components including a light source such as a light emitting diode ("LED") or laser, a light beam modulator, mirror, lenses, photo diodes, sample holders and other optional components, as further described herein. In any event, the sample holder provides for easy insertion of membrane test strips, with a minimal amount of pass through of ambient or stray light, thus reducing the noise level. A reading device having an improved signal to noise ratio is provided, with greater sensitivity. The sample holder may include a mechanical design having a spring-loaded member. In some applications, at least two different stop positions are provided for the same membrane test strip wherein the first stop position may be used to provide a reference reading, and a second stop position

may be used to read actual samples in a detection area or a detection zone.

One embodiment of the invention is further illustrated in Figure **2**, wherein a reading device **40** receives a membrane strip **41** into a receiving port **45** to provide a result. A light barrier structure **28** also is shown. A detection zone **42** upon the membrane strip **41** is located some distance from a reference zone **43**, which gives a base line reference or calibration reading. In the particular embodiment shown, the detection zone **42** is provided towards the outside, while the reference zone **43** is towards the inside, but it should be recognized that the positions of these respective zones could be reversed from that which is shown in Figure **2**.

The reading device **40** may include a housing exterior **44**, and on/off switch **49**, and housing interior (not shown in Figure **2**). In Figure **2**, an LCD display **60** is shown.

In Figure **3**, the light barrier structure **28** is shown in a view with the components exploded upwards from the housing exterior **44** of the reading device **40**. The top plate **50** is also shown. The device shown in Figure **3** corresponds to the device shown in Figure **2**, and is essentially the same embodiment. The receiving port **45** is bounded on its lower edge by bottom plate **56**, and on its upper edge by top plate **50**. Within the receiving port **45** there is a pressure plate **51**, under which the membrane strip **41** is inserted. The pressure plate **51** is held by spring

52 in a resilient engagement with the membrane strip **41** (not shown in Figure **3**). The membrane strip **41** is held over aperture **54**, which happens to be circular in Figure **3**. However, the aperture could be of many different shapes and sizes, and most preferably approximates the size and/or shape of the zone of interest upon the membrane strip **41** that is to be examined. The channel **53** forms the conduit through which the membrane strip **41** is inserted. Screws **55a-d** holds the top plate **50** down upon the housing **44**.

In Figure **3a**, the underside of top plate **50** is shown, revealing a recess **58**. Within the recess **58** resides the pressure plate **51**, which is held in springing engagement by spring **52**. Also shown is a light-absorbing member **57**, which rests upon the top or upper surface of membrane strip **41** (see Figure **2**). The light-absorbing member **57** acts as a low reflectance specimen in contact with the aperture **54** that allows the instrument to be calibrated to eliminate the effects of internal reflections within the sensor housing. In practice, such calibration can be performed automatically by the microprocessor when power is first applied to the instrument. Furthermore, the light absorbing member **57** may absorb any light which is transmitted completely through the membrane strip **41**, so that such light is not reflected back downward towards the sensor **92** (see Figure **5a**). In this way, the sensitivity and signal to noise ratio of the reading device **40** is maximized.

The light-absorbing member **57** may include almost any type of material that is capable of absorbing light, such as a black or dark colored flocking, plastic, metal, felt, or other material. For example, materials that are used in the photography arts that are known to absorb light could be employed. Such materials may be flexible and/or conformable, and may be comprised of felt. There is no particular size or shape that is preferred for a light-absorbing member **57**, but it is important that the light-absorbing member **57** cover completely the area under which the membrane strip **41** is being impacted by light from its underside. One optional feature of the light-absorbing member **57** would be to provide a flexible or conformable form fit to the test strip, by using felt or drapable material.

Figure **4** shows a cross section of the light barrier structure **28** with receiving port **45** as shown in lines **4-4** of Figure **2**. The receiving port **45** comprises a pressure plate **51** that fits between a top plate **50** and a bottom plate **56**. A membrane strip **41** is inserted below the pressure plate **51**, where the detection zone **42** of the membrane strip **41** may be placed directly over a light pathway **59**. Light generated by a light source (now shown in Figure **4**) such as a light emitting diode (LED) passes upwards along arrow **59a** and is reflected downward from membrane strip **41** along arrow **59b** as seen in Figure **4**.

The internal light emitting and sensing components of the reading device shown in Figures **2-4** is essentially the same as that shown in Figures **5-5a**.

It is important to the sensitivity of the reading device **40** that the light aperture located immediately below the membrane strip **41** is of a size that approximates the size of detection zone **42** upon the membrane strip **41**. In other applications, the aperture (not shown in Figure **4**) may be slightly larger than the detection zone **42**. In some cases, the aperture could be about 1.3 or even 1.8 times larger in area than the detection zone **42**. However, it has been found that the closer the aperture corresponds to the size of the detection zone **42** upon the membrane strip **41**, the higher the signal to noise ratio that can be achieved by the reading device **40**, and the more sensitive will be the reading device **40**. Furthermore, the membrane strip **41** also may include a reference zone **43** at another location upon the membrane strip **41**. The reference zone **43** may be placed over the light pathway **59** in order to obtain a reference reading or a calibration of the reading device **40**. Then, in a second step, the detection zone **42** may be placed over the light pathway **59** to obtain the sample reading. A spring **52** is shown in cross section above the light-absorbing member **57**, which fits just above the membrane strip **41**. The light-absorbing member **57** is capable of absorbing light that may undesirably enter the receiving port **45** from outside. Furthermore, the light-absorbing member **57** is capable

of absorbing light that may proceed through the light pathway **59**, and be transmitted completely through the membrane strip **41**. This prevents reflection downward of stray light, improving sensitivity.

One alternate embodiment of the invention is shown in Figure **5**. A light barrier structure **81** is provided, below an LCD display **74**. The light barrier structure is bounded from above by top plate **72**, and from below by bottom plate **78**. A reading device **65** is comprised of a housing **73** having a receiving port **64** bounded upon the top by a hood **66**. The receiving port **64** consists in part of a channel **68** that runs vertically as shown in Figure **5**. An aperture **69**, (which in Figure **5** happens to be in the shape of a rectangle) is located in the bottom of the channel **68**. A first notch **70** and a second notch **71** are provided as locating points to receive a membrane strip having nub **77** which will be seen in Figure **5a**. Screws **67a** and **67b** hold the hood **66** down upon the top plate **72**. The function of the hood **66** is to reduce the amount of ambient light that impacts near the aperture **69**, increasing the sensitivity of the reading device **65**, and improving the signal to noise ratio of results obtained. An on/off switch **75** is shown near the right side of the housing **73**.

Figure **5a** is a basic schematic taken in cross section along lines **5a-5a** of Figure **5** showing the basic internal architecture of the reading device **65** employed in the invention. Screws **67a-b** hold down a top plate **72** upon bottom plate **78,** and also function to hold hood **66** to plate

72. In cross section, one can see a light-absorbing member **80** that is positioned above membrane strip **76**. A nub **77** fits into first notch **70** to register the membrane strip **76** in the appropriate position to receive light **91** from a light emitting diode (LED) **90**. The light **91** travels to the membrane strip **76**, and then is reflected downward along light pathway **93** to a sensor **92**. In some applications, the sensor **92** is a diode. A housing **73** is also seen, and may include other components that are not shown in Figure **5a**.

A basic schematic diagram of a reading device **65** is shown in Figure **5b**. In Figure **5b**, an LCD display **74** having 16 characters is shown on the right side of Figure **5b**. The LCD display **74** is connected to a micro controller **95**. The microcontroller **95** directs the activities of the reading device **65**, and regulates the light energy output of the light emitting diode (LED) **90**, as shown in the lower left portion of Figure **5b**.

Likewise, a photo diode **92** receives light energy, and converts such energy to signals that are transmitted to a preamplifier **79,** and then to the microcontroller **95**. Eventually, the data output or result of an assay is illuminated on the LCD display **74**, shown in Figure **5**.

The wavelength of the illumination radiation should be chosen to fall within the wavelength range over which the detector (photodiode) has appreciable responsivity (typically 400 nm to 1000 nm for a silicon photodiode. Furthermore, the wavelength of the illuminating radiation

should be chosen to be near the maximal absorption wavelength of the detectable material used as the label in the lateral flow assay.

It is generally accepted that the detectable material used as a label or probe in the assay is one that will interact with light in the visible or near visible range, by absorption. For example, if the probe is a substance that appears blue to the naked eye when concentrated, the ideal electromagnetic radiation would likely be yellow. Particulate direct labels, including metallic and gold sols, non-metallic elemental sols (i.e. selenium or carbon) and colored latex (polystyrene) particles are suitable examples, as further described herein.

The source of light represented by the light emitting diode **90** may be comprised entirely of commercially available components. Suitable examples are commercially available LED's, preferably chosen to provide a suitable wavelength of light that is strongly absorbed by the detectable material concentrated in the detection zone **42**. If desired, an array of LED's, which are energized in turn, could be used.

Figure **6** shows a more detailed view of the top plate **72** of one embodiment of the invention, which is seen in Figure **5**. A membrane strip **76** having a nub **77** is registered into first notch **70** as shown. In some embodiments of the invention, the nub **77** registers with the first notch **70** to take a reading from a reference zone **83** on the membrane strip **76**. Then, once a reference or calibration reading is obtained, the membrane strip **76** may be lifted up and the position changed so that the

nub **77** is integrated into the second notch **71**. A detection zone **82** is shown on membrane strip **76**. The detection zone **82** would then be placed over the aperture (aperture is not shown in Figure **6**) to obtain the test sample reading. The channel **68** into which the membrane strip **76** is placed is shown in Figure **6**.

Figure **7** shows a cross sectional view along lines **7-7** of Figure **6**. Screws **67a-b** holds the hood **66**, and a top plate **72** to a bottom plate **78**. A membrane strip **76** is provided in the channel **68**, so that the nub **77** is fitted into first notch **70**. The light-absorbing member **80** is positioned over the membrane strip **76** in Figure **7**. The light-absorbing member **80** may include those materials described for component **57**, including almost any type of material that is capable of absorbing light, such as a black or dark colored flocking, felt, plastic, metal, or other material.

The membrane-based device of the invention comprises several components, including a membrane, a sample pad, a conjugate pad and a wicking pad, or a combination of these items. The membrane typically includes at least two zones, that is, one or more detection zone(s) and one or more control or reference zone(s). A sample pad contacts one end of the conjugate pad.

One design of the assay device includes a liquid sample flow direction having a sample pad, conjugate pad, detection zone, and a pad, typically provided in that order from one end to the other end. In general, the wicking pad assists in promoting capillary action and fluid

flow one-way through the membrane strip. The pad "pulls" the liquid containing the analyte along the membrane from one end of the membrane to another end of the membrane.

Probes used in the invention may comprise beads or particles. Such beads or particles may be comprised of latex, or other suitable material, as further described herein. In some applications, plain particles are used, while other applications may employ particles with capture reagents and/or antibodies conjugated upon the outer surface of the particle. The particles are typically colored with a dye that is visible to the eye, or to a detection apparatus. In other embodiments, the particles may include light absorbing materials such as metal sols, gold, or silver particles. Gold nanoparticles have been found to be suitable in some applications.

In one application of the invention a system for conducting a lateral flow assay is provided to detect the quantity of analyte that resides in a test liquid. The system comprises employing a probe analyte conjugate complex that is capable of generating a detectable signal. Furthermore, a membrane strip is provided and configured for mobilizing a test liquid which contains both a probe and an analyte conjugate. The membrane strip comprises a detection zone, in which the detection zone has deposited thereon a first capture reagent. The first capture reagent is immobilized upon the detection zone, and is configured for attaching to probe analyte conjugates to immobilize the

probe analyte conjugates, thereby forming a sandwich complex within the detection zone.

A detection line may contain an immobilized second capture reagent (i.e.: antibody or other conjugating species), which serves to immobilize the unbound probes by binding to form a control probe complex (i.e.: immobile species) on a capture line. When significant numbers of the probe are immobilized in this way, a visibly distinctive line appears at one or more detection lines on the membrane strip. The control line may be embedded with a predetermined amount of second capture reagent.

In some instances, a comparison is made between the intensity levels of the calibration or control lines (or zone), or some other reference standard, and the detection line of the membrane strip, to calculate the amount of analyte present in a sample. This comparison step is accomplished with the reading device further described herein.

The membrane strip employed in the assay may be a cellulose ester, with nitrocellulose usually providing good results, but the invention is not limited to such compositions for the membrane strip.

It is to be understood that the invention can be configured for detecting a broad range of analytes, including therapeutic drugs, drugs of abuse, hormones, vitamins, glucose proteins (including antibodies of all classes), peptides, steroids, bacteria or bacterial infection, fungi, viruses, parasites, components or products of bacteria, allergens of all types,

antigens of all types, products or components of normal or malignant cells, and the like.

The following analytes are examples of analytes that may be tested using the present invention: T.sub.4, T.sub.3, digoxin, hCG, insulin, theophylline, luteinizing hormone, organisms causing or associated with various disease states, such as streptococcus pyogenes (group A), Herpes Simplex I and II, cytomegalovirus, chlamydiae, and others known in the art.

United States Patent No. 4,366,241 (Tom et al.) lists at columns 19-26 a variety of potential analytes of interest that are members of an immunologic pair, including proteins, blood clotting factors, hormones, microorganisms, pharmaceutical agents, and vitamins. Any of these analytes are suitable for use as the analyte in present invention.

Other examples of preferred ligands or analytes that may be detected include the following: human bone alkaline phosphatase antigen (HBAPAg); human chorionic gonadotropin (hCG); human luteinizing hormone (hLH); human follicle stimulating hormone (hFSH); creatine phosphokinase MB isoenzyme; ferritin; carcinoembryonic antigen (CEA); prostate specific antigen (PSA); CA-549 (a breast cancer antigen); hepatitis B surface antigen (HBsAg); hepatitis B surface antibody (HBsAb); hepatitis B core antigen (HBcAg); hepatitis B core antibody (HBcAb); hepatitis A virus antibody; an antigen of human immunodeficiency virus HIV I, such as gp 120, p66, p41, p31, p24 or

p17; the p41 antigen of HIV II; and the respective antiligand (preferably a monoclonal antibody) to any one of the above ligands. The HIV antigens are described more fully in United States Pat. No. 5,120,662 and in Gelderblood et al., Virology 156: 171-176 1987, both of which are incorporated herein by reference.

As used herein, the term "probe" refers generally to a structure that is capable of carrying an analyte in a lateral flow assay to a detection area or zone, which may or may not be in the form of a particle or microparticle. Furthermore, as used herein the term "probe-conjugate" refers to a species that is capable of carrying an analyte in a lateral flow assay to form a probe-conjugate complex, which binds a first capture reagent in a detection zone of a membrane strip to become a "sandwich complex" in the detection zone.

As used herein, the term "microparticle" is a more specific reference to a particular type of probe, and may include any beads or probes to which an antibody may be bound, whether covalently, or non-covalently such as by adsorption. An additional requirement for some particles that are used in a quantitative assay is that the particle contributes a signal, usually light absorption, which would cause the zone in which the particles were located to have a different signal than the rest of the membrane.

Optionally, metallic particles or metal could be used as the probe in the invention. These particles are commercially available as

microspheres of substantially uniform diameter from companies such as British Biocell International, of Cardiff, United Kingdom.

By the phrase "membrane" or "membrane strip" as used herein is meant a test device or strip that employs a membrane and one or more reagents to detect the concentration of an analyte of interest in a test solution, preferably an aqueous test solution. At least one of the reagents associated with the membrane device is a binding partner of the analyte of interest.

Latex microparticles for use in the present invention are commercially available as polymeric microspheres of substantially uniform diameter (hereinafter "polymeric microspheres"), such as from Bangs Laboratories of Carmel, Indiana, or Dow Chemical Co. of Midland, Michigan. Although any polymeric microsphere that is capable of adsorbing or of being covalently bound to a binding partner may be used in the present invention, the polymeric microspheres typically are composed of one or more members of the group consisting of polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutyleneterephthalate, acrylonitrile, vinylchloride-acrylates and the like or an aldehyde, carboxyl, amino, hydroxyl, or hydrazide derivative thereof.

The underivatized polymeric microspheres, such as polystyrene,

are hydrophobic and passively adsorb other hydrophobic molecules, including most proteins and antibodies. Techniques for adsorbing a protein or polypeptide on a hydrophobic particle are provided in the publication by Cantarero, et al. "The Absorption Characteristics of Proteins for Polystyrene and Their Significance in Solid Phase Immunoassays," Analytical Biochemistry 105, 375-382 (1980); and Bangs, "Latex Immunoassays," J. Clin. Immunoassay, 13 127-131 (1980) both of which are incorporated herein by reference.

Various procedures for adsorbing molecules on polymeric microspheres are also described, in general terms, in Bangs, L. B., "Uniform Latex Particles," presented at a workshop at the 41st National Meeting, Amer. Assoc. Clin. Chem., 1989, and available in printed form from Seragen Diagnostics Inc., Indianapolis, Ind.; or Galloway, R. J., "Development of Microparticle Tests and Immunoassays, " i.e., Seradyn Inc. of Indiana which is incorporated herein by reference.

The test solution may be a component of a biological fluid, such as extracted, diluted, or concentrated from a plant or animal, preferably a mammal, more preferably a human. Especially preferred biological fluids are serum, plasma, urine, ascites fluid, peritoneal fluid, amniotic fluid, synovial fluid, cerebrospinal fluid and the like, or a concentrate or dilution thereof.

In the practice of the invention, calibration and sample testing may be conducted under essentially exactly the same conditions at the same

time, thus providing highly reliable quantitative results, and increased sensitivity.

The invention also may be employed for semi-quantitative detection. As the multiple control lines provide a range of signal intensities, the signal intensity of the detection line can be compared (i.e. such as for example, visually) with the control lines. Based on the intensity range the detection line falls, the possible concentration range for the analyte may be determined. The probes may be latex beads labeled with any signal generating species or the labeled latex beads further conjugated with antibodies.

It is understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention, which broader aspects are embodied in the exemplary constructions. The invention is shown by example in the appended claims.